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BIOLOGICAL BULLETIN

MISCELLANEOUS BIOLOGICAL OBSERVATIONS ON BUMBLEBEES.[1]

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Although the biology of bumblebees has been a favorite study of numerous amateur and professional naturalists, many questions concerning the life-history and habits of these interesting insects are still unsolved. In several recent papers (1922 *a*, *b*, *c*; 1923 *a*, *b*), I have discussed some of these questions, and in the following paragraphs wish to touch briefly upon several others which, because of their heterogeneity, could not be incorporated in the earlier papers.

I. CONDITION OF PILE AND NESTING HABIT.

In his monograph on the bumblebees of Thuringia, Schmiedeknecht (1878) expresses the belief that loss of thoracic pile in bumblebees indicates that the colonies to which such individuals belong have subterranean nests. That this theory is incorrect is shown by the following observation. On July 6, 1921, I transferred a surface nest of *Bremus* (*Bombus*) *fervidus* to an observation-box in which the colony was kept until the first part of October. At the time the nest was taken, the pile of all the members of the colony, including that of the queen, was in perfect condition, and remained that way until the latter part of July, when the dorsal side of all the foraging workers became coated with a layer of yellow pollen. These layers of pollen gradually hardened and sometimes reached several millimeters in thickness. The bees evidently were annoyed by these lumps of pollen, for they repeatedly tried to rub them off with their front legs. Whenever they succeeded in doing so, most of the pile on the dorsal side of the thorax came off with the pollen,

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 226.

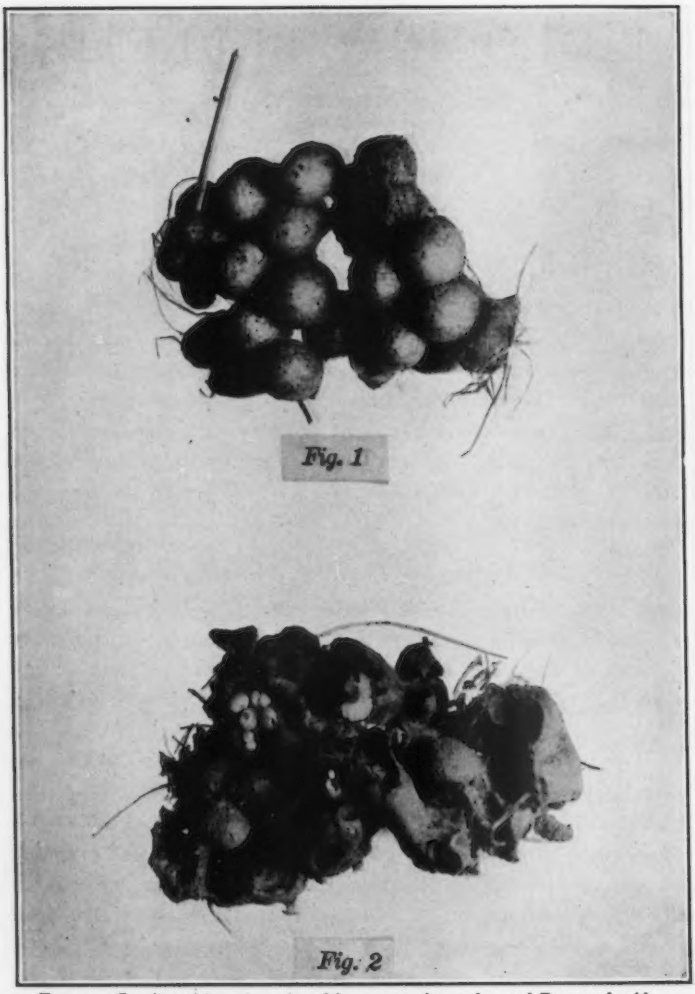

FIG. 1. Comb and brood produced by a queenless colony of *Bremus fervidus* Fabricius.

FIG. 2. Comb and brood produced by a queenless colony of *Bremus impatiens* Cresson.

leaving the center of the mesonotum completely bare. Similar observations were made on several *Bremus fervidus* colonies during the summer of 1922. Investigation showed that these lumps of pollen were acquired by the bees while they were gathering nectar from *Linaria*, popularly known as butter and eggs, or toad flax. In the light of these facts, it is not surprising that Schmiedeknecht's (pp. 384, 424) predictions in regard to the nesting habits of some of the rarer European species have proved to be incorrect (cf. Gundermann, 1908, p. 31). This is also true of some of the predictions of Hoffer (cf. Härter, 1890, pp. 71–73).

II. The Structure of the Outer Nest.

Wagner (1907) states that the fibrous envelope of epigeic bumblebee nests is composed of two distinct layers which he calls the outer and the inner nest. Although I have examined a large number of epigeic nests belonging to several American and European species, I have never found an indication of such an arrangement, and this leads me to believe that such nests as those shown by Wagner (pp. 31–32) in Figs. 14, 15, and 16, instead of being of universal occurrence, are extremely rare.

III. The Population of a Bumblebee Colony at Night.

Packard (1865), Coville (1890), and Sladen (1912) state that the whole population of a bumblebee colony is at home during the night, while Shuckard (1866), whose observations agree with those of Hoffer (1882/83), has the following to say concerning this matter: "If, in their collecting excursions, they are intercepted by heavy rains, or loiter far away too long until twilight closes, they will pass the night away from home, and return laden with their gatherings as soon as the warmth of the sun reanimates them to activity; thus they will often sleep in flowers, and a nest therefore taken at night is not always a sure indication in those found within it, of its complete population." For many years, I also held the same opinion as Packard (p. 109), Coville (p. 198), and Sladen (pp. 47, 99), but during the summer of 1921, I accidentally discovered that the observations of Shuckard (p. 315) and Hoffer (p. 5) are correct.

IV. Size of Colony and Nest Cavity.

Wagner (1907, p. 36) states that the size of the nest cavity of species which nest in the ground corresponds to the size of the colony. In reply to this assertion, I may state that during the summers of 1921 and 1922 I dug up a number of incipient bumblebee colonies whose nests filled less than half of the nest cavity.

Although he has never actually observed this, it is further claimed by Wagner (pp. 36, 153–154) that bumblebees excavate, or at least enlarge, subterranean cavities for their nests. Sladen (1912, p. 40), on the other hand, has the following to say concerning this question: "As the larger and newer cocoons become available for storage of food, the oldest ones at the bottom of the comb are emptied and used no more, except in a time of plenty, when all the rest are full. In underground nests, where all available space is likely to be needed for the expansion of the comb, the walls of these abandoned cocoons are often bitten down, and the comb sinks. Wasps, it is well known, enlarge their nest cavity according to their requirements by digging out little lumps of earth and flying away with them, but I have never seen bumblebees do this." To this statement, which agrees with my own observations, I may add that the fragments of such dismantled cocoons are sometimes found intermingled with the material, *e.g.* dry grass, which with the bees cover the comb.

V. The Proximity of Bumblebee Colonies.

Some years ago, Wagner (1907) discovered a colony of *Bremus muscorum*, and about 35 cm. from it a destroyed bumblebee nest. This led him to conclude that the members of the colony had left the old nest and had built a new one, either because their larvae had been destroyed by parasites or because they had been robbed of their comb, for bumblebee colonies, according to Wagner (p. 157), never nest in such close proximity. In reply to this assertion of Wagner (1907), I may state that I recently (June 26, 1922) discovered a colony of *Bremus impatiens* and one of *Bremus vagans* which had their nests within two feet of each other, and, judging from my experience with colonies in artificial nests, there seems to be no reason why bumblebees could not nest in even closer proximity.[1]

[1] On July 29, 1923, shortly after the manuscript of this paper had been mailed

VI. The Defense of the Nest.

Wagner (1907) claims that one, or a small number of bumblebees, even if they belong to a populous colony, never attack, and that the courage of a bumblebee depends upon the number of individuals present. This certainly is not true of species like *Bremus fervidus*, *impatiens*, *lapidarius*, and *terrestris*. On more than one occasion I have been attacked with great ferocity by single workers of these species at the old nest-site, while the remaining members of the colony were miles away. Equally incorrect, as Hoffer (1888, pp. 97–98), Sladen (1912, pp, 254, 277), and I (1922 *a*, p. 34) have shown, is Wagner's claim (pp. 144–145) that the members of a bumblebee colony do not coöperate in expelling strange bumblebees from their nest.

As can easily be demonstrated, the temper of a bumblebee colony depends upon a number of factors. If, for instance, the colony is chilled, even savage species, *e.g. Bremus fervidus*, exhibit little or no fighting spirit. This lack of temper becomes still more marked if, in addition, the colony has been compelled to fast. The ferocity of a bumblebee colony, to a certain extent, also depends upon previous experience. If the nest of a savage species, like *Bremus fervidus*, is frequently disturbed, certain members of the colony will attack any one whom they find in the vicinity of their nest. It is probable, therefore, that Wagner (pp. 151–152), at least in the case of *Bremus lapidarius* and *terrestris*, based his conclusions on observations which were made at a time when the mettle of his colonies was low as a result of adverse conditions.

VII. Incubation of the Brood.

There is a difference of opinion among bumblebee students as to why bumblebees incubate their brood. Hoffer (1882/83, I, p. 14) has the following to say concerning this activity: "Die Königin liegt dabei häufig so über der Zelle, dass sie dieselbe mit dem Bauche wärmt, wie eine Henne ihre Eier, wobei der Bauch dicht an die Zelle angedrückt wird. Dieses Bebrüten übt sie übrigens auch an älteren Eier- und Larvenklumpen und den Puppentönnchen aus." A similar view is held by Sladen (1912,

to the editor, I took two flourishing bumblebee colonies—one of *Bremus fervidus* and the other of *Bremus pennsylvanicus*—which had their nests within a foot of each other.

pp. 25–31). However, this explanation is rejected by von Buttel-Reepen (1903), who suggests that, on the contrary, bumblebees, being guided solely by pleasant stimuli in such cases, probably incubate their brood because they profit by the warmth which is given off by the larvæ. Several well-founded objections to von Buttel-Reepen's (pp. 38–39) theory have been published by Wagner (1907). Among other things, Wagner (pp. 93–95) has pointed out that bumblebees incubate objects from which they cannot possibly get warmth, a statement which is corroborated by my own observations. Thus, for example, I have frequently seen queens and workers of various species incubate the outer rim of tin cans which were used in artificial breeding experiments, even when the thermometer was below the optimum temperature for bumblebees. While external stimuli undoubtedly play an important part in this interesting habit of bumblebees, broodiness probably also depends upon a certain physiological condition. Only in this way, it seems to me, can one explain the fact that it sometimes takes several weeks until a confined *Bremus* queen becomes broody, and that, after becoming so, she readily incubates various objects, such as tin cans or the floor and sides of her nest-box, in some cases even days or weeks before she lays her first eggs.

VIII. Red Clover and Pollen.

In his monograph on bumblebees referred to at the beginning of this paper, Schmiedeknecht (p. 328) claims that bumblebees cannot obtain pollen from clover. This statement likewise is incorrect. The *corbiculæ* of bumblebees working on red clover almost always contain dark-brown pollen, and by a half hour's observation one can easily convince oneself that this pollen is obtained from the clover.

IX. Method of Depositing Pollen.

Another point on which there is a difference of opinion, is the question as to how a bumblebee deposits her load of pollen. Coville (1890), whose observations agree with those of Sladen (1912), describes this activity as follows: "If pollen-laden, the bee balanced herself, with her middle and cephalic pairs of legs, on the edge of a pollen-pot, head outward, spread her wings, and then scraped the pollen-masses from her *corbiculæ* by rubbing the

posterior legs together." Hoffer (1882/83, I, p. 16), Wagner (1907, p. 98), and Frison (1917), on the other hand, state that the bumblebee removes the pollen with the middle pair of legs, and this agrees with my own observations. Frison (pp. 283–284) describes the process as follows: "After the pollen-containing cocoon has been found, the worker stands on the edge facing away from the cocoon, and inserts her hind legs down into the cocoon; then she proceeds by a quick, slicing downward movement of the middle pair of legs, to remove the pollen from the *corbicula*." It is difficult to conceive how bumblebees could deposit pollen in the manner described by Coville (p. 200), and Sladen (p. 46), and it seems probable therefore that these two authors overlooked the rapid movement of the second pair of legs.

X. On the Behavior of Pollen-laden Bumblebees in Late Fall.

Wagner (1907) claims that bumblebees suffer a great reduction of instincts toward the end of the breeding season. As one of the proofs for this assertion, he states that in late fall pollen-laden bumblebees return to the nest, but leave again without depositing their pollen. Although I had about 75 bumblebee colonies under observation during the summers of 1921 and 1922, I was unable to observe any such behavior, and this leads me to believe that there were other reasons than those given by Wagner (p. 190) why his bees left without depositing their pollen.

XI. The Capping of Honey Cells.

Hoffer (1882) and Sladen (1912), the two foremost European authorities on the biology of bumblebees, differ as to whether bumblebees cap their honey. Hoffer (I, p. 39) has the following to say concerning this question: "Ist das Honigtöpfchen voll, so wird sehr häufig ein stumpf-kegelförmiger Deckel darüber gebaut, der aber in der Mitte eine ganz kleine Oeffnung hat, so dass also niemals vollständig gedeckelte Honigzellen angetroffen werden, wie sie die Bienen haben." Sladen (p. 53), on the other hand, says: "In a favourable season a populous colony may have all the vacated cocoons, amounting to over 400, filled with thick honey and sealed over with wax." Observations made on *Bremus ternarius* (by Putnam, 1865), *Bremus auricomus* (by Frison, 1917),

and *Bremus impatiens* (by myself) in this country, confirm those of Sladen (1912).[1] In large colonies of *Bremus impatiens*, I have repeatedly found a considerable portion of the lower cocoons filled with thick honey and completely capped so that no opening could be seen, even with the aid of a hand lens.

XII. The Food of Bumblebee Larvæ.

In his extensive paper on the hive-bee,[2] Swammerdam (1752) also refers incidentally to some of his biological observations on bumblebees, and expresses the belief that the larvæ of the latter feed on the wax by which they are surrounded, since, in his opinion, it seems improbable that the adults open the wax covering to feed the larvæ. But Huber (1802) found that this is precisely what the adults do, and that the food of the larvæ consists of a mixture of pollen and honey, and not of wax. These observations of Huber (pp. 263–265) were later confirmed by Schmiedeknecht (1878, p. 321) and Hoffer (1882/83). However, several years later, Härter (1890), being unable to observe any such method of feeding, questioned the correctness of Hoffer's (p. 27) observations and came out strongly in support of Swammerdam's (pp. 194–195) hypothesis. Since, to my knowledge, Härter's (p. 67) claim has never been denied, I should here like to mention the further positive evidence by Coville (1890) and Sladen (1912) whose observations agree with my own. In this connection, it may be stated that the Pocket-makers, as was first pointed out by Sladen (1899), use a somewhat different method of feeding their larvæ, at least those of the workers. This question will be discussed more fully in connection with another paper.

XIII. Assistance to Emerging Young.

Huber (1802), Dahlbom (1832), and von Buttel-Reepen (1903) state that bumblebees assist their young during the process of hatching by widening the slit which the emerging bee makes in its cocoon. Not long ago, Wagner (1907) subjected this question to the experimental test. He isolated some cocoons of *Bremus lapidarius* and found that the first worker which emerged—although two days old—did not pay the slightest attention to the

[1] Since writing the above, I have discovered that the French Abbé Noël de Pluche (1764) also made this observation.

[2] This paper (cf. Swammerdam, p. vi) was completed in 1673.

second when the latter was struggling to leave its cocoon. From this rather superficial experiment, Wagner (p. 108) infers that bumblebees do not assist their young during the process of hatching. However, against this negative evidence of Wagner (1907), we have the further positive evidence of Sladen (1912), Bachmann (1915), and Frison (1917), whose observations I have been able to confirm.

XIV. The Adoption of Individuals from Other Colonies

Wagner (1907, p. 172) claims that after a bumblebee has been in the nest of another species from 10 to 12 hours she is, without exception, treated as a member of the colony. This statement, as well as Wagner's (p. 172) assertion that the specific odor of a bumblebee colony emanates only from the queen, is disproved by the results of several breeding experiments which I carried out during the summer of 1922 (cf. 1923*a*). These experiments show that in some cases even members of such closely related species as *Bremus fervidus* and *Bremus pennsylvanicus* may refuse to have anything to do with each other, even after they have been together for weeks, and although the members of one of these species have been reared by the other.

XV. The Homing Instinct of Queens and Workers.

Wagner (1907) states that young bumblebee queens take a much longer orientation flight than the workers, and that despite this fact they have much more difficulty in finding the nest than the latter. I have observed the first orientation flight of a large number of young queens and workers belonging to several species, and although I found considerable individual variation in the aptitude which these bees showed in finding the nest, I failed to note any such correlation as that described by Wagner (pp. 218–219). In fact, if any generalizations can be made, I should say that the young queens appeared to have less difficulty in finding the nest than the workers.

XVI. Natural Requeening.

In the works of Hoffer (1882/83), Handlirsch (1888a, 1888b, 1891), Sharp (1899), Friese and Wagner (1904, 1910, 1912, 1914), Vogt (1909, 1911), and Franklin (1912/13), one occasionally

meets with the statement that the members of a bumblebee colony are the offspring of one queen, and on this "fact" several of the above-mentioned authors have based far-reaching conclusions in regard to the genetic constitution of certain species. However the observations of Putnam (1865), Lindhard (1912), Sladen (1912), and Frison (1921) indicate that this is by no means always the case, since queens which have started colonies are often killed and supplanted by others which enter their nest, a fact which is corroborated by my own observations (cf. 1922*c*, 1923*a*). Here in the vicinity of Boston, this natural requeening is especially common with *Bremus fervidus* and *Bremus impatiens*.

XVII. Nests Established by Queenless Workers.

Wagner (1907) believes that a bumblebee colony which has lost its queen and comb cannot build another nest, and hence is unable to contribute anything toward the next generation. Huber (1802, pp. 288–289), on the other hand, states that bumblebee workers which have lost both queen and comb are able to start a new nest.[1]

In order to determine which one of these two authors is correct, the following experiments were carried out. On August 1, 1922, I removed all the comb from a queenless colony of *Bremus fervidus* which at that time consisted of about 100 workers. The latter at once started to build a new nest and produced a considerable number of males. Two weeks later, the same experiment was performed with a colony of *Bremus impatiens* consisting of about 300 workers, and this yielded the same result. The accompanying plate shows how the comb of the two colonies looked on August 21, 1922. These experiments prove conclusively that Wagner's (pp. 116–117, 154–155) contention is incorrect.

XVIII. The Efficiency of Bumblebees.

Wagner (1907) claims that bumblebees are so inefficient in their work, especially that connected with nest-building, that 8/9 of their efforts are wasted. I cannot agree with Wagner (pp. 136–154) on this point. While it is true that bumblebees do not produce such populous colonies as certain other Social Hymen-

[1] Wheeler and Taylor (1921) have found that this is also true of social wasps.

optera, they are, as the following observation shows, nevertheless very industrious and efficient creatures. On August 5, 1921, I placed about 50 workers belonging to a large colony of *Bremus impatiens* in a glass-covered box and provided them with honey (in a porcelain dish) and five pieces of old bumblebee comb, each consisting of about a dozen empty cocoons. The bees started at once to repair three of the pieces of comb with wax which they took from the other two. In the course of a few hours, nothing remained of these two pieces of comb except a few shreds, and the remaining three were completely renovated and filled with honey. The rapidity with which this work was carried out shows that Wagner's (1907) claim is an exaggeration.

XIX. On the Hibernation Instinct of Bumblebees.

Wagner (1907) claims that the psychic qualities of social insects are no higher, and in some cases even lower, than those of solitary insects. As proof for this contention he cites (p. 196) the "fact" that the queens of bumblebees and social wasps overwinter singly, while certain solitary bees, *e.g. Xylocopa*, hibernate in groups. That these assertions of Wagner (pp. 195–196) are incorrect, at least as far as social wasps are concerned, is shown by recent observations of Mr. and Mrs. Rau (1918), who found that the queens of some of our North American wasps congregate for hibernation; and, as the following observations indicate, there also seems to be a tendency in this direction in bumblebees. In artificial overwintering experiments, Lindhard (1912) found as many as eleven *Bremus* queens hibernating close together, and I was able to make similar observations out in the open. On October 15, 1922, I dug up—within an area of less than two square feet—21 queens of *Bremus impatiens*, many of which were hibernating in groups of two and three. In dealing with this question, Wagner (p. 196) furthermore overlooked the fact that bumblebee colonies in certain parts of the tropics are probably perennial (cf. von Ihering, 1903).

Equally incorrect is Wagner's (p. 9) surmise that a spot free from grass constitutes one of the conditions for the young bumblebee queen in the choice of her hibernaculum, for the spot in which the 21 queens were hibernating was completely covered with grass.

XX. The Position of the Nest and Hibernation.

Wagner (p. 7) states that it depends upon the fall weather when bumblebees begin to hibernate, and that queens reared in well-protected nests (*e.g.* those in the ground or in hay stacks) take up their winter quarters later than those reared in surface nests. These two statements are likewise incorrect. In Europe, the queens of *Bremus pratorum*, a species which frequently nests in the ground (cf. Armbruster, 1914, p. 686), begin to hibernate in July (cf. Sladen, 1912, p. 12), while those of such surface-nesting species as *agrorum* and *muscorum* do not begin to hibernate until at least a month later. The same thing holds true for some of our American species. Here in the vicinity of Boston, the nests of *Bremus bimaculatus* and *Bremus terricola* are almost always subterranean, and yet the majority of the queens of these two species take up their winter quarters in July, while those of *Bremus fervidus* and *Bremus vagans*, species which usually nest on the surface of the ground, do not begin to hibernate until the latter part of August. These facts indicate that the beginning of hibernation depends on the species rather than on the weather and the position of the nest.

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THE FUNCTIONS OF THE SWIMBLADDER OF FISHES.

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INTRODUCTION.

The swimbladder, or air-bladder, of a fish is situated dorsal to the cœlom, between the alimentary canal and the vertebral column. It is a membranous sac containing the atmospheric gases. On a portion of the surface of the bladder there is a vascular and glandular area, known as the *rete mirabile*, or "gas gland," or "red gland." Embryologically the swimbladder arises as a diverticulum from the dorsal side of the alimentary canal. The primitive connection may be permanently retained as a tubular canal, called the *ductus pneumaticus*, or it may be entirely absent in the adult. Because of this variation the teleostean fishes have been grouped into two divisions—the Physostomi, characterized by the retention of the duct, and the Physoclisti, in which the duct is absent in the adult. In the former group are included the carp, salmon, and eel; in the latter, the cod, bass, and perch. The basis for this distinction between the two groups, however, is far from invariable and many exceptions occur in both.

The variety of functions performed by the swimbladder is perhaps greater than that of any other organ possessed by fishes.

Among the important functions assigned to the swimbladder are: (1) phonation, or sound producing; (2) respiration; (3) accessory audition; (4) hydrostatic activities.

The respiratory function is believed by most scientists to be important only in a few species. The sound-producing function also has been demonstrated in a limited number of species. Apart from the function of phonation, the swimbladder occasionally becomes subservient to the auditory organ, with which it may be connected directly or through the interpolation of certain modified parts of the anterior vertebræ. The most important function of the swimbladder is hydrostatic, by virtue of which a fish possesed of such an organ is able to alter the amount of contained air and consequently its own specific gravity, so as to be in equilibrium with the surrounding medium under varying pressures.

The investigations to be described in this paper began during the summer of 1921 while the author was in the employ of the United States Bureau of Fisheries. From results obtained that summer and from suggestions gained from Dr. A. S. Pearse, it was thought worth while to continue the study of the functions of the swimbladder. Pearse (1920) had previously shown that the swimbladder gases change in percentage, oxygen becoming less, when perch are kept in water containing small amounts of dissolved oxygen. An attempt was first made to ascertain how important this fact might be in explaining how fishes are able to go into regions where the oxygen is extremely low or entirely absent and live for several hours. The problem developed from that work and was finally extended to cover other functions which the swimbladder might perform. The experiments were restricted to yellow perch, *Perca flavescens* (Mitchell); large mouth black bass, *Micropterus salmoides* (Lacépéde); and carp, *Cyprinus carpio* (Linnæus); A few comparisons were made with other species inhabiting Lake Mendota.

Historical.

The rôle that the swimbladder plays in the life of fishes has attracted the attention of investigators for a long time. Aristotle believed the organ to function chiefly in the production of sounds. Borelli (1680) was perhaps the first to attribute to the swimbladder

an hydrostatic function. He maintained that the fish possessed a volitional control over the size of the swimbladder—being able to compress or distend the bladder at will. Delaroche (1807) opposed the views of Borelli but advanced an hypothesis similar to it in many respects. Moreau (1877), however, maintained that he had disproved both these hypothesis. Delaroche was among the first to show that an exchange of gas probably occurred between the swimbladder and the blood. He did not consider the former an organ of respiration however.

Biot (1807), Provencal and Humboldt (1809) showed by analysis of the air in the swimbladder that the mixture frequently consisted almost entirely of oxygen, the percentage of oxygen increasing in relation to the depth of the water in which fishes lived. Moreau (1877) in his classical work of the function of the swimbladder proved by ingenious experiments that many of the ideas prevailing before his time were erroneous. He showed that this organ serves to equilibrate the body of the fish with the surrounding water at any level. He demonstrated that such adjustment is not accomplished quickly, and that the fish, therefore, does not use his muscles in regulating the volume of the swimbladder. Moreau's experiments also convinced him that the gas is secreted into the swimbladder. About the same time Johannes Müller (1842) took up the problem. Although many of his views have later been shown untenable, they served as an incentive to other investigators. He stated that the gas entered the swimbladder by secretion from the blood.

Several views have been held concerning the origin of gases in the swimbladder. The different theories can be classified into two distinct categories: (1) Those that hold that the gases are derived directly from the atmosphere, attributed first to Redi (1684); (2) and those that maintain that these gases are derived more or less directly from the blood stream (a descendant of Needham's secretory theory).

The first view has been given up by most modern investigators, although Thilo (1906) has persistently contended that the blood of fish is not sufficient in quantity to supply the amount of gas found in the bladder. He asserts that in all cases the bladder-gas must be procured by the fish directly from the atmosphere. Objections are easily found to Thilo's hypothesis.

For example the percentage composition of the three gases, nitrogen, oxygen, and carbon dioxide, is quite different in the swimbladder from that present in the atmosphere. Thilo does not explain how the degeneration of the ductus pneumaticus in the Physoclisti can be correllated with their ability to change their specific gravity.

The second class of theories contains three distinct views as to how the bladder-gases are derived from the blood stream: (1) those which suppose that the blood gives up its gaseous constituents more or less directly to the bladder, the gas passing directly from the blood into the bladder lumen; (2) those that suppose that the gas gland is a pumping organ that is able to force gases from the blood into the swimbladder gland; and (3) those that hold that the gases of the swimbladder are derived from the cytoplasmic decomposition of the gas gland cells.

The first view which is often associated with the name of Moreau (1877), is now quite out of date, although references are often made to it in textbooks and other works of general nature. The second and third views are still current and have excited much controversy. Both of these suppose that the gas gland is the special organ which extracts the gas from the blood. They have been held for several years with great tenacity by schools represented by Jaeger, and opposed by the adherents of Nusbaum and Reis.

Jaeger (1904, 1908) holds that the gas gland is primarily a pumping apparatus, an apparatus for pumping the gases contained in the blood into the swimbladder cavity. He further supposes that the disintegration of a certain percentage of red blood corpuscles is effected by secretions from the gas gland cells —a toxin being poured into the blood for this purpose.

Nusbaum (1907) and Reis (1906) as a result of extensive studies of the cytology of the gas gland have formulated certain peculiar views as to its functions. They contend that gas bubbles are produced by actual decomposition of the substance of the gas gland cells, just as gases are produced by putrefaction. They also believe that erythrocytes are taken into the cells of the gas gland and decomposed, oxygen being liberated and used for such decomposition. There are many objections to such a theory. For example, nitrogen is not known to be formed by

decomposition and yet a large percentage of the swimbladder air is usually nitrogen.

Woodland (1911) attempted to compromise the Jaeger-Nusbaum controversy. He assumed that a lysin was secreted by the gas gland cells. This he supposed to hemolyse the erythrocytes and thus liberate oxygen which the gas gland cells could hold and thus force into the swimbladder lumen. Woodland refuted his own hypothesis in the following year however (1912) and concluded "that hemolysis does not occur in the gas gland," but he offered no suggestion as to how gas does enter the swimbladder. It is obvious that a more or less continual hemolysis or a disintegration of the erythrocytes would be a severe drain on the blood, and it seems probable that some other explanation, more economical for the organism, could be found. The writer attempts to furnish such an explanation in the present paper.

General Methods.

In the study of the functions of the swimbladder of fishes reported in the following pages the attempt was made to employ standard biochemical and physiological methods. In many cases, however, special apparatus was needed. Such apparatus was devised and is described in connection with the respective experiments.

In the analyses of the composition of the gases within the swimbladder, the Haldane (1912) gas analysis apparatus was used; the Newcomer (1919) modification being found to be the most convenient form. A burette holding five cubic centimeters was used instead of the one with the usual ten cubic centimeter capacity, for the reason that the quantity of gas obtainable from the swimbladder is, in most of the fishes used, less than ten cubic centimeters. The sample was drawn from the fish into a five cubic centimeter sampling tube (Fig. 1.) The sampling tube *S* connected with a levelling tube *L* and a hypodermic needle *H* were filled with mercury. The hypodermic needle was thrust through the body wall directly into the swimbladder. The stopcocks were opened, the levelling tube lowered, and the gas thus drawn into the sampling tube. The stopcocks were then closed. The samples were introduced into the gas analysis apparatus the same day and analyzed in the usual manner.

The methods for determining gases in water were similar to those described by Birge and Juday (1911). Many of the experiments were conducted in the field in order to study the fishes in their natural habitats. Samples were taken from a boat and although at first this seemed somewhat inconvenient, it was later found that little difficulty accompanied the operations. Temperatures, soundings, etc., were recorded at the time the experiments were performed.

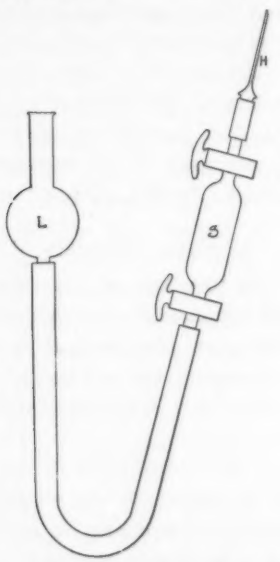


FIG. 1. Swimbladder gas collecting tube.

THE NORMAL COMPOSITION OF GASES IN THE SWIMBLADDER.

It was of course important to know the normal composition of the gases in the swimbladders of the species under investigation. The gases vary with the species, with the dissolved gases in the surrounding media, with varying temperatures and pressures, and apparently with other factors that are not yet understood. Probably, also, the degree of salinity of the water, the season of the year, and the physiological condition of individual fishes (influenced directly or indirectly by factors already mentioned) act to produce variations in the composition of the gases found normally in the swimbladder of any particular species.

An attempt was made to study the normal composition of the gases of the swimbladder of several species of fishes under as nearly natural conditions as could be obtained. Fishes were caught in gill nests and traps and were immediately conveyed and transferred to a wire cage sunk near the surface of the water in Lake Mendota. The location of the cage was previously tested for its suitability. The fishes were left in this cage from 48 to 72 hours. Samples of air content of the swimbladder were then taken by methods already described. The amounts of dissolved gases, temperature, and depth, were recorded at the time the samples were taken. The results are summarized in Tables I. and II.

TABLE I.

NORMAL PERCENTAGES OF THE GASES OF THE SWIMBLADDER OF PERCH AFTER BEING KEPT IN A WIRE CAGE FOR 72 HOURS IN LAKE MENDOTA. SEPTEMBER, 1921.

No.	Date.	Gases in Water.		Depth, Meters.	Temp. ° C.	Gases in Swimbladder.	
		O_2.	CO_2.			Per Cent. O_2.	Per Cent. CO_2.
20	1	4.8	−2.53	.6	24.8	17.4	0.60
21	1	"	"	"	"	21.5	0.66
22	1	"	"	"	"	15.6	0.46
23	1	"	"	"	"	16.8	0.60
32	4	4.8	−2.53	.6	24.8	12.9	1.15
33	4	"	"	"	"	25.1	0.77
34	4	"	"	"	"	21.8	1.03
35	4	"	"	"	"	22.3	0.96
36	4	"	"	"	"	9.5	0.44
37	4	"	"	"	"	23.0	0.62
38	5	4.6	−1.01	.6	22.0	28.0	0.86
39	5	"	"	"	"	22.6	0.69
40	5	"	"	"	"	17.4	0.53
41	5	"	"	"	"	19.8	0.65
42	5	"	"	"	"	22.0	0.68
43	5	"	"	"	"	21.2	0.66
44	5	"	"	"	"	22.0	0.62
45	6	5.8	+2.20	.6	23.0	18.8	0.63
46	6	"	"	"	"	24.0	0.58
47	7	"	"	"	"	25.4	0.45
48	7	"	"	"	"	17.2	0.60
49	7	"	"	"	"	15.2	0.66
50	7	"	"	"	"	20.8	0.39
51	7	"	"	"	"	25.2	0.62
52	7	"	"	"	"	23.5	0.43
53	7	"	"	"	"	24.8	0.43
54	7	"	"	"	"	22.6	0.64
55	7	"	"	"	"	10.3	0.34
Averages		5.1	0.30	.6	23.4	19.9	0.63

TABLE II.

THE RESULTS OF ANALYSES OF THE SWIMBLADDER GASES OF VARIOUS SPECIES OF FISHES SEINED IN UNIVERSITY BAY, LAKE MENDOTA, NEAR THE SURFACE, SEPTEMBER 12–14, 1921.

The dissolved gases ranged as follows: Oxygen = 3.8–4.0 c.c. per liter; Carbon dioxide = 1.7–2.0 c.c. per liter. The temperature was 22.2° C.

Species.	No.	Gases in Swimbladder.	
		O_2.	CO_2.
Pickerel	1	42.9	2.10
Blue-gill	1	14.8	0.48
Sunfish	1	17.6	0.60
	2	24.2	0.56
	3	22.1	0.28
	4	21.2	0.81
	5	22.2	0.49
	6	24.3	0.71
	7	21.9	0.68
	8	20.9	0.67
	9	23.5	1.07
Largemouth black bass	1	19.2	0.52
	2	22.4	1.00
	3	25.7	0.86
Rock bass	1	20.4	0.86
	2	21.5	1.06
	3	21.2	0.31
	4	21.3	0.97
	5	20.4	0.64

At different times during the year, fishes were taken from tanks in the laboratory and the gas content of their swimbladder analyzed for the purpose of controls for various experiments. The tanks were supplied with water pumped from Lake Mendota. The water was well aerated before it was introduced into the tanks, because that coming directly from the pipes was supersaturated with atmospheric gases. The fishes were kept for long periods of time in these tanks in apparently good condition, although for most of the experiments fishes were used that had been brought in from the lake within a period of from 10 to 14 days. The results of the analyses of apparently normal fishes taken from laboratory tanks are summarized in Table III.

It is shown that the normal compositions of gases in the swimbladder of perch was 19.9 per cent. oxygen and 0.63 per cent. carbon dioxide when the fishes were kept in a normal environment.

TABLE III.

THE RESULTS OF ANALYSES OF THE SWIMBLADDER GASES OF VARIOUS FISHES KEPT IN LABORATORY TANKS.

The temperature varied from 20 to 23 degrees centigrade. August and September.

Species.	Dissolved Gases c.c./liter.		Gases in Swimbladder.	
	O_2.	CO_2.	Per Cent. O_2.	Per Cent. CO_2.
Small mouth Black Bass ...	5.5	N	18.9	22.3
Sucker.....	6.3	N	5.4	4.35
Carp.....	4.93	4.5	4.2	2.5
	4.93	4.5	3.8	3.1
	4.93	4.5	2.6	3.7
	4.93	4.5	3.2	2.8
	5.5	N	7.3	5.4
	5.5	N	12.7	5.7
	5.5	N	7.6	4.5
	5.5	N	7.9	4.3
	4.6	N	5.3	5.8
	5.4	N	4.9	3.0
	5.3	N	1.8	0.8
	3.9	.76	7.4	3.3

THE EFFECTS OF OXYGEN DEFICIENCY.

Pearse (1920) showed that perch, even though they are able to recognize the proportion of oxygen and carbon dioxide in water, enter regions where conditions are unfavorable for respiration and may remain there in oxygen-free water for as much as two hours without dying. He also showed that the oxygen supply of the swimbladder was depleted when the fishes were subjected to waters of low oxygen content. On his suggestion further investigations were carried on during the summer of 1921.

A search was made, in Lake Mendota, for a region of low oxygen. Such an one was found at the mouth of University Creek, in University Bay. This creek empties into the southern end of the bay in shallow water behind a bar. During the summer months a thick covering of duckweed (*Lemna* and *Wolffia*) may be found there. The position of this plant covering is governed by the direction of the wind, but whenever a wind from the North, Northwest, or Northeast is blowing, or when there is little or no wind, one may prophecy with a fair degree

of assurance that the mouth of University Creek will be covered with a mat of this duckweed which prevents the processes of photosynthesis from going on below. Thus a region of very low oxygen is produced.

A wire cage, three feet high, three feet wide, and three feet in length, was sunk in this region. The experimental fishes were placed in this cage, having been previously kept in a cage of similar dimensions in regions of more normal oxygen content. The fish were left in the cage from one to two hours as recorded in Table IV., which shows the results of the experiments.

Another series of experiments was carried on in the laboratory. A large still of thirty-five gallons capacity, loaned by the Wisconsin State Prohibition Commissioner, was used for obtaining oxygen-free water. The water was brought to the boiling point and siphoned through a series of condensers. The water was conveyed directly from the condensers to an aquarium which was kept at the same temperature as the water in which the fish were accustomed to live. Fishes were placed in this aquarium and removed at intervals. The gaseous content of the swimbladder and the amount of dissolved gases in the water were determined from samples taken simultaneously. The results of these experiments are shown in Table V. Control fishes were kept in aquaria at the same temperature and where other conditions were as similar as possible to that in the aquaria where fishes were subjected to low oxygen. The swimbladder analyses of ten controls averaged 16.0 per cent. oxygen and 0.74 per cent. carbon dioxide.

The results of these experiments show that when perch were subjected to water of low oxygen content in Lake Mendota averaging 2.1 cubic centimeters per liter, the percentage of oxygen decreased, and was 12.1 per cent, as compared with the normal of 19.9 per cent. When perch were subjected to water containing extremely small amounts of oxygen, averaging 0.67 cubic centimeters per liter, in aquaria in the laboratory, the percentage of oxygen in the swimbladder decreased and was 9.78 per cent. as compared with normal controls having 16.0 per cent. oxygen.

These results lead one to speculate concerning to what extent the ability of the fishes to re-absorb oxygen from the swimbladder

explains how such fishes are able to go into regions of low oxygen content and live for several hours. The question can be answered tentatively by certain theoretical considerations. A normal perch of average size has a swimbladder capacity of

TABLE IV.

ANALYSES OF THE GASES OF THE SWIMBLADDERS OF PERCH SUBJECTED TO WATERS OF LOW OXYGEN CONTENT, IN A WIRE CAGE IN LAKE MENDOTA. SEPTEMBER 7 TO 15, 1921.

No.	Gases in Water.		Depth, Meters.	Temp., ° C.	Gases in Swimbladder.	
	O_2.	CO_2.			Per Cent. O_2.	Per Cent. CO_2.
56	0.85	+15.4	0.7	22.0	17.4	1.37
57	"	"	"	"	17.2	1.63
58	"	"	"	"	16.5	1.20
59	"	"	"	"	11.0	1.17
60	"	"	"	"	15.4	1.36
61	"	"	"	"	18.2	2.45
62	"	"	"	"	19.0	2.04
63	1.9	+11.6	"	21.0	14.0	1.79
64	"	"	"	"	9.8	1.57
65	"	"	"	"	16.3	1.25
66	"	"	"	"	12.2	2.93
67	"	"	"	"	7.3	1.00
68	"	"	"	"	11.3	0.89
69	"	"	"	"	10.8	1.03
70	"	"	"	"	9.5	1.37
71	1.45	+10.1	"	21.8	13.8	2.04
72	"	"	"	"	21.3	1.76
73	"	"	"	"	12.8	1.86
74	"	"	"	"	13.8	2.00
75	"	"	"	"	21.7	1.89
76	"	"	"	"	15.7	2.62
92	1.6	+10.1	0.8	19.1	6.3	1.47
93	"	"	"	"	15.5	0.73
94	"	"	"	"	11.1	1.81
95	"	"	"	"	9.0	1.39
96	"	"	"	"	8.0	1.49
97	"	"	"	"	7.9	1.41
98	"	"	"	"	8.8	1.23
99	"	"	"	"	9.2	1.21
100	"	"	"	"	11.9	2.03
109	4.1	+12.6	"	18.0	14.5	1.48
110	"	"	"	"	8.7	1.05
111	"	"	"	"	8.0	1.37
112	"	"	"	"	7.0	1.28
113	"	"	"	"	7.6	1.19
114	"	"	"	"	12.7	1.48
115	"	"	"	"	5.0	1.24
116	"	"	"	"	7.5	1.43
117	"	"	"	"	8.6	1.44
Averages	2.1	+11.9	0.74	20.2	12.1	1.54

TABLE V.

ANALYSES OF THE GASES FROM THE SWIMBLADDERS OF PERCH SUBJECTED TO WATER OF LOW OXGYEN CONTENT.

Experiments were performed in a laboratory aquarium. Water made oxygen-free by boiling and fishes were left in it for varying periods of time. *N* indicates normality, *i.e.*, no free carbon dioxide.

No.	Date, 1922.	Dissolved Gasses in Water.		Temp., ° C.	Time, Hrs.	Gases in Swimbladder.	
		O_2.	CO_2.			Per Cent. O_2.	Per Cent. CO_2.
1	March 20	0.7	*N*	7.0	5	9.8	1.40
2	" 20	0.7	*N*	7.0	5	6.9	1.34
3	" 26	1.0	*N*	6.0	6	12.2	2.08
4	" 26	1.0	*N*	6.0	10	16.8	2.30
5	April 1	1.1	*N*	6.0	5	13.3	2.40
6	" 1	1.1	*N*	6.0	8	18.8	5.40
7	" 2	0.8	*N*	6.0	6	5.4	2.50
8	" 2	0.8	*N*	6.0	9	15.4	2.40
9	" 12	0.6	*N*	7.0	5	9.4	0.22
10	" 13	0.7	*N*	7.0	6	7.8	0.62
11	" 16	0.7	*N*	7.0	3	16.4	0.92
12	" 16	0.7	*N*	7.0	3	8.0	0.31
13	" 16	0.7	*N*	7.0	4	14.9	0.25
14	" 16	0.7	*N*	7.0	4	5.4	0.12
15	" 17	0.3	*N*	7.0	4	11.2	0.38
16	" 17	0.3	*N*	7.0	4	2.85	0.12
17	" 17	0.3	*N*	7.0	4	5.9	0.55
18	" 18	0.3	*N*	7.0	4	2.8	0.14
19	" 18	0.3	*N*	7.0	6	1.72	0.78
Averages....		0.67	*N*	6.7	5.3	9.73	1.27

approximately ten cubic centimeters. The average percentage of oxygen was found to be 19.9 per cent. In other words the actual amount of oxygen would be about two cubic centimeters. From work on the rate of respiratory exchange in perch (to be reported in a later paper) the oxygen consumption was found to be 61.8 cubic centimeters per kilogram of weight per hour at 3° C. The average perch weighs somewhat less than 100 grams. Thus the amount of oxygen required would be about six cubic centimeters of oxygen per hour. The amount that the swimbladder could furnish is only a fraction of that amount. We must conclude, therefore, that although the perch may draw on the swimbladder for oxygen when in regions of low oxygen, the amount that can be furnished to the blood by re-absorption is

not enough to keep the fish alive for more than a fraction of an hour. Some other explanation must be found for the reason why fishes are able to survive for longer periods of time in regions of low oxygen content.

Effects of Pressure.

That pressure would affect the percentage of gases in the swimbladder of fishes has been long known. Biot (1807), Provencal and Humboldt (1809) found that the amount of oxygen in the swimbladder varied from 1 to 87 per cent., the percentage increasing with the depth. The classical work of Moreau (1842) showed that the fish accommodate themselves to changes in pressure very gradually, and can live comfortably at varying depths.

Only a few experiments are reported here to show that the response of perch to pressure results in the same increase in oxygen as reported by other investigators for other fishes. It was observed that fishes caught in gill nets at a depth of nine meters in Lake Mendota showed a much higher oxygen percentage than those caught near the surface. The averages of nineteen perch caught at nine meters, during month of August, 1921, were:

Oxygen = 34.7 per cent., Carbon dioxide = 0.60 per cent.,
Nitrogen = 64.7 per cent.

The averages of twenty-eight perch kept at the surface in Lake Mendota were:

Oxygen = 19.9 per cent., Carbon dioxide = 0.63 per cent.
Nitrogen = 79.5 per cent.

From the data of Birge and Juday (1911) the amount of dissolved oxygen at this season of the year was less at nine meters depth than at the surface. Thus it appears that the cause of this increase in the amount of oxygen in the swimbladder can be attributed to pressure.

An apparatus was constructed in the laboratory to study the effects of pressure as a factor in producing increase in the percentage of oxygen in the swimbladder, other conditions being equal. A steam pipe, eight inches in diameter and thirty-six

inches long, was closed at one end and fitted with a cap at the other end. In this cap was fitted a glass window four inches in diameter through which the fish could be observed. Pipes running from the fourth floor of the Biology Building were connected with this tank, one carrying water to the tank, one carrying water away from the tank. The height of the overflow pipe was approximately sixty feet above the tank. Thus a pressure was obtained in the tank equal to that at a depth of sixty feet in the lake, or at about two atmospheres greater than at the surface. The fish could be very quickly removed and samples taken for analysis.

Six controls that were kept in water containing the same amounts of dissolved gases and at the same temperature as the fishes subjected to pressure showed the following averages for gases in their swimbladders:

Oxygen = 12.1 per cent., Carbon dioxide = 0.65 per cent.,
Nitrogen = 87.5 per cent.

The averages for seven perch under sixty feet of water pressure at the end of ten hours duration were:

Oxygen = 18.5 per cent., Carbon dioxide = 0.50 per cent.,
Nitrogen = 81.0 per cent.

This shows that with an increased pressure the percentage of oxygen is increased.

The Effects of Increased Carbon Dioxide.

Carbon dioxide is found in very small amounts in the atmosphere (0.08 per cent.). It is found more abundantly in water on account of its ready solubility. The amount in the water, however, is governed by the partial pressure of the gas in the atmosphere. The free carbon dioxide dissolved in natural waters is affected by photosynthesis and by organic decomposition.

Carbon dioxide is also found in natural waters in chemical union. It is found in two states (Birge and Juday, 1911): that united with a base in the form of normal- or mono-carbonate such as $CaCO_3$ or $MgCO_3$ and is known as "fixed" or "combined" carbon dioxide; and that which converts the monocarbonate

into a bicarbonate, called "half-bound" or "bicarbonate" carbon dioxide. The half-bound is of course not in such stable union as the fixed. Although plants can make use of the bicarbonate for photosynthesis it can be of very little importance to fishes. It is only the free carbon dioxide that is considered in the following experiments.

The determination of carbon dioxide is not especially accurate when done by titration methods and can not always be relied upon for absolute results. Probably the most accurate method is that of Pettenkofer's described and modified by Birge and Juday (1911). Such a method was used in these experiments.

Carbon dioxide has a marked effect upon fishes. As in other animals the physiological effect is that of a narcotic, stimulating in small quantities and intoxicating in larger quantities. Carbon dioxide and acids apparently produce similar effects when judged by the behavior of fishes. Reuss (1910) working with varying amounts of carbon dioxide upon rainbow trout found that the effects were similar to those upon birds and mammals. Shelford (1918) also showed that when individuals of several species where dropped into water containing 168 cubic centimeters of carbon dioxide per liter, the first effect was one of stimulation but in a few minutes fishes lost their power to perform correlated movements and died. Shelford also showed that fish display a preference for low carbon dioxide and that they react very definitely to amounts naturally found in ponds and lakes. He concludes that the carbon dioxide content of the water is the best single index of the suitability of the water for supporting fishes.

A study was made of the effects of carbon dioxide in the surrounding water on the gases in the swimbladders of perch. The experiments were carried on in the laboratory. Carbon dioxide gas was bubbled through a large carboy of water. A constant flow of water was kept through the carboy to an aquarium containing the experimental fishes. Individual fishes were removed at intervals and analyses made of the swimbladder gases. Water samples were taken at the same time. All the experiments were begun by having normal lake water in the aquarium and gradually increasing the amount of treated water. The results are shown in Table VI. In every case the oxygen and carbon

dioxide in the swimbladder both increased as the carbon dioxide in the surrounding water increased.

TABLE VI.

ANALYSES OF THE SWIMBLADDER GASES OF PERCH SHOWING THE EFFECTS OF AN INCREASED AMOUNT OF CARBON DIOXIDE IN THE WATER.

The perch were placed in an aquarium when the water was at the neutral point. The carbon dioxide was gradually increased and the amount recorded represents the amount dissolved in the water at the time a fish was removed for analysis.

No.	Gases in Water.		Temp., ° C.	Time.	Gases in Swimbladder.	
	O_2.	CO_2.			Per Cent. O_2.	Per Cent. CO_2.
1	10.1	+ 13.7	8.5	3 hrs.	15.6	1.06
2	10.4	+ 21.5	10.0	6 "	12.7	1.72
3	11.7	+ 35.4	10.0	9 "	17.7	1.64
4	12.4	+102.5	9.0	12 "	15.3	7.15
5	11.1	+126.5	9.0	14 "	16.4	10.40
6	11.9	+ 55.7	14.0	2 "	15.8	4.08
7	16.5	+ 72.3	12.0	4 "	14.8	5.80
8	16.5	+ 93.5	11.5	6 "	15.6	6.60
9	16.6	+117.0	11.0	8 "	14.8	8.05
10	17.5	+110.0	11.0	10 "	21.1	5.90
11	7.2	+ 43.0	12.0	3 "	16.1	2.25
12	7.2	+ 60.0	12.0	4 "	17.9	4.30
13	7.6	+ 83.5	12.0	5 "	18.1	5.25
14	7.6	+ 83.5	12.0	6 "	19.7	6.0

It was observed during the preceding experiment that fishes would rise in the aquarium as the carbon dioxide was increased, and this suggested that the specific gravity of the fish perhaps changed. It may also be seen that the oxygen percentage remained fairly constant, indicating that the volume was apparently increasing. An apparatus was devised to determine if the volume of the fish did actually increase. A diagram of the apparatus is shown in Fig. 2. A large bottle *C* of three liters capacity was sealed with a rubber stopper *R*; a three-way stopcock *S* and capillary tubing connected the bottle with a tambour *T*. The tambour controlled a marker which recorded on a smoked drum *D* any change in volume that might take place within the bottle. The bottle was immersed in a constant temperature water bath *B*. The bottle was filled with water having the same temperature as that of the constant temperature bath which was of the same temperature as the water in

which the fish was accustomed to live. This water contained a normal amount of oxygen. The apparatus was tested several times to make sure that no leak occurred and that no change in volume took place when fishes and normal water were in the bottle or when water containing large amounts of carbon dioxide and no fish was used. There was no indication of any volume

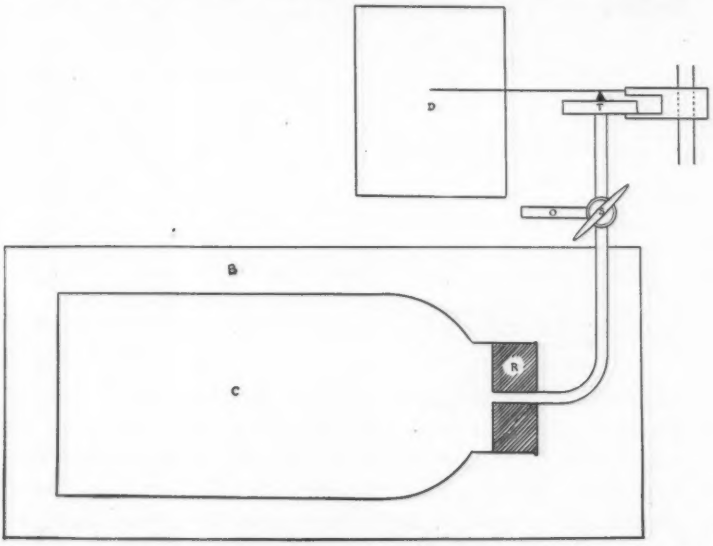


FIG. 2. Apparatus for determining the volume change in fishes. *C*, large mouth bottle; *R*, rubber stopper; *S*, three way stopcock; *T*, tambour; *D*, drum; *B*, constant temperature water bath.

change in any of these tests. Individual perch were placed in the bottle, the water having previously been charged with a small amount of carbon dioxide, the rubber stopper pushed tightly into the aperture; the water rose and passed out through the tube *O*, the stopcock was turned so as to connect the bottle with the tambour. The drum was started and a record of the volume change made.

A typical graph is shown in Fig. 4. It will be observed that the most rapid changes took place within the first few minutes. Is this response of the fish to carbon dioxide due to the acid nature of the dissolved gas or to some other property? It is evidently not due to the acid property, for when lactic acid is

used instead of carbon dioxide the volume of the fish does not increase, but on the contrary shows a decrease. Fig. 6 shows a graph obtained when lactic acid was added to the water (1:1000). The effects produced by the lactic acid is probably one of constriction of tissues caused by the acid; the effect of carbon dioxide is probably due to diffusion of the gas into the swimbladder.

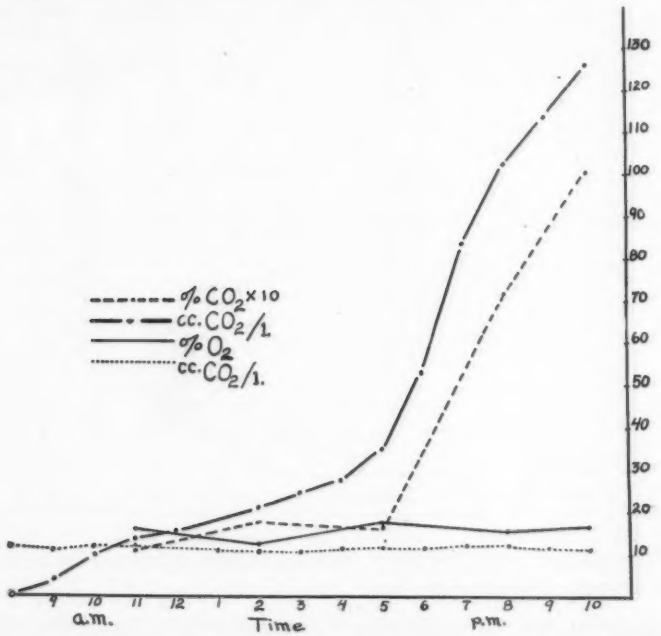


FIG. 3. A graph to show comparisons of the dissolved gases in the water with the gases in the swimbladder with variation of the dissolved gases. The swimbladder gases are expressed in percentages and the dissolved gases in cubic centimeters per liter. A single experiment is represented in the graph. Perch were placed in an aquarium containing normal lake water and the carbon dioxide gradually increased. The time intervals are expressed on the abscissa and the amounts of the gases expressed on the linear scale of the ordinate.

The fact that fishes change their volume when subjected to water containing high amounts of carbon dioxide may be of ecological importance. In many lakes the amount of carbon dioxide dissolved in the water increases with depth. The oxygen decreases with depth and near the bottom may be

entirely lacking. Both these factors make the bottom of a lake less favorable for respiration than shallower regions. Of course a high amount of carbon dioxide is toxic and the lack of oxygen may lead to asphyxiation. In Fig. 6, the average carbon

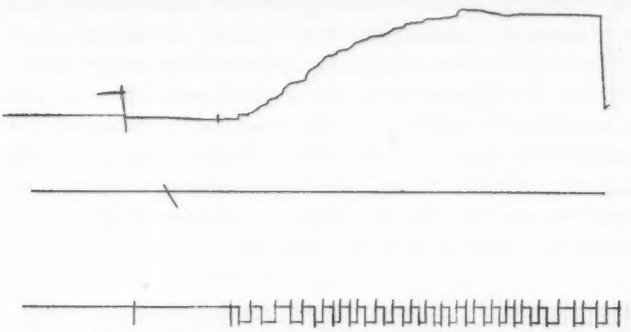


FIG. 4. A graph showing the volume change of a perch when placed in water where dissolved free carbon dioxide was increasing. Time is recorded at two-minute intervals.

dioxide gradient of Lake Mendota is plotted for the month of August from 1906 to 1921. The data were obtained from records kept by President Birge and Professor Juday. It can be seen that the carbon dioxide gradually increases with depth and averages about five cubic centimeters per liter at the bottom.

FIG. 5. A graph showing the temporary decrease in volume when a perch was placed in water containing lactic acid (1 : 1000). Time is recorded at two-minute intervals.

At times it may be two or three times as great as this average figure for Lake Mendota. Such an amount of carbon dioxide may be sufficient to produce an increased volume in the fish and cause t to rise above regions that might be unfavorable for respiration. Shelford (1913) has shown that dissolved carbon dioxide may be taken as an index for the distribution of fishes. He attributes distribution to the results of behavior-regions

high in carbon dioxide being avoided by the fishes. From the experiments described in the present paper, it may be assumed that such distribution is due, at least in part, to a more or less automatic mechanism which operates so as to control vertical distribution by altering specific gravity. Both these processes are ordinarily adaptive and may operate simultaneously.

The results of these experiments show that when fishes are subjected to increased amounts of dissolved carbon dioxide in water, the carbon dioxide in the swimbladder increases and is roughly proportional to that in the water. Fig. 4 illustrates this point. Carbon dioxide produces an alteration in the specific gravity of the fish, causing it to rise and escape from regions which might be unfavorable for its existence.

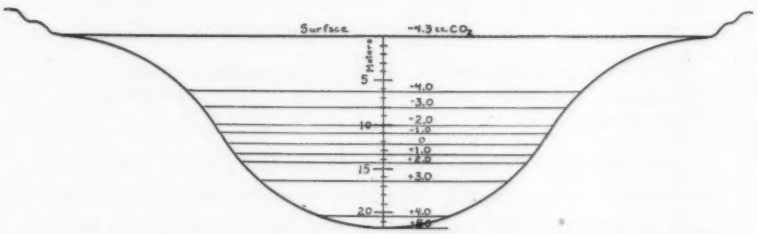


FIG. 6. A diagram to show the carbon dioxide gradients with depth in Lake Mendota for the month of August from 1906–1920. The data were obtained from Birge and Juday. The minus sign indicates alkaline water and the plus sign acid.

DIFFUSION AND SECRETION.

A fluid gives off gas to or takes gas from any other medium with which it is in contact according to the relative pressure of the gases. Dalton's *Law of Partial Pressures* may be stated as follows: in a mixture of gases each gas exerts the same pressure as it would exert if it were alone present in the volume occupied by the mixture. The pressure of each gas is called its partial pressure.

It is believed by most physiologists that the inter-change of gases during respiration is explained by the laws of physical diffusion. The interchange of gases in the lungs has raised a problem that has inspired considerable study. While many physiologists have accepted the view that during the interchange of gases in the lungs, the membranes between the blood and the

alveolar air simply play a passive part, and physical diffusion explains the respiratory exchange. However, this view has not gone unchallenged.

Haldane and Smith (1896) claims that the tension of the oxygen in the arterial blood may be higher than the pressure of oxygen in the alveoli. According to the physical theory of respiration, if a permeable membrane separates two volumes of any gas, or two solutions of any gas at different pressures, the molecules of the gas will pass through the membrane in both directions until the pressure is equal on both sides; *i.e.*, gas diffuses from a point of high rension to one of low tension. Haldane maintains that a secretory activity is associated with the function of the lungs, which produces a higher tension of oxygen in the arterial blood than in the alveolar air.

The analogy has many times been drawn between the gas interchange in the lungs and in the swimbladder of the fish. Bohr (1894) punctured the swimbladder and removed gas. He found that if the fish was then left in the water, the gas was rapidly replaced, and, when he tapped the swimbladder a second time, that the percentage of oxygen had greatly increased. Oxygen may amount to between 60 and 80 per cent of the total gas after such an experiment. Bohr found that this reaccumulation did not take place if both vagi were cut and he ascribed it therefore to direct secretory activity on the part of the swimbladder. Bohr is inclined to endow the vagus nerves of the higher vertebrates, including mammals, with an analagous regulatory influence on the gaseous exchange in the lungs.

Starling (1920) strongly maintains that the lungs cannot be considered analagous to the swimbladder. He says there is no likeness between the thick secreting cells of the "red gland" which is apparently the gas-secreting part of the swimbladder, and the thin structureless plates which separate the capillaries of the lungs from the alveolar air.

It was considered necessary to repeat some of Bohr's work during the present experiments, using fresh-water fishes. Yellow perch were used for the first series. Gas samples were obtained by methods already described (p. 000). After the first sample was taken the fishes were placed in the same tank in which they had been accustomed to live. No changes in the amounts of

dissolved gases were detected in the water during the experiments A second gas sample was taken from the swimbladder after a varying interval of time. A comparative record of the results are shown in Table VII.

The large black bass and the carp were also used and the results are summarized in Table VII.

TABLE VII.

RESULTS OF EXPERIMENTS WITH THE SECOND REMOVAL OF GASES FROM THE SWIMBLADDER; THE DISSOLVED GASES IN THE SURROUNDING MEDIUM WERE KEPT CONSTANT.

Species.	No.	Gases in Swimbladder, First Removal.		Duration of Time between First and Second Removal in Hours.	Gases in Swimbladder, Second Removal.	
		Per Cent. O_2.	Per Cent. CO_2.		Per Cent. O_2.	Per Cent. CO_2.
Smallmouth black bass.	1	18.9	2.22	24	36.2	4.02
	2	36.2	4.02	24	46.0	3.20
	3	18.7	1.15	48	46.0	5.54
Largemouth black bass	1	20.0	1.48	24	29.7	1.72
	2	18.7	0.72	24	30.0	3.06
	3	17.9	0.88	24	31.3	2.66
Yellow perch.....	1	13.1	0.70	12	16.1	1.57
	2	11.1	0.60	12	35.2	3.10
	3	12.7	0.60	12	23.1	1.70
	4	14.0	0.85	12	25.2	2.00
Carp.....	1	4.2	2.5	7	4.8	1.45
	2	3.8	3.1	7	4.8	0.60
	3	8.0	4.3	48	10.7	3.16
	4	5.4	5.1	24	5.7	5.10

The results show that the percentage of oxygen increases in the bass and the perch when second sample is taken after an interval of time. In the carp, however, only slight changes could be observed. The carp has an open duct leading from the swimbladder while the bass amd the perch have closed swimbladders. This is believed to be the explanation for the difference in the response of the two types to withdrawal of air from the swimbladder.

It is apparent that the question of whether oxygen is transferred to the swimbladder by physical diffusion or secretion rests on a knowledge of the partial pressures of the gases in the blood and in the swimbladder. If one can know the partial pressures

or tensions of the oxygen in the swimbladder and in the blood coming to the swimbladder he will have the solution of the problem. If the tension of the gases should be the same in the swimbladder and in the blood coming to the swimbladder one could but conclude that the gases were transferred by physical diffusion, but if the tensions were not equal and should be markedly greater on one side than on the other he would be justified in concluding that an active secretion existed.

However, the determination of the gaseous tensions in the blood presents considerable difficulty. It is necessary to bring the blood in contact with gaseous mixtures containing various proportions of the gas whose tension in the blood it is desired to measure. By making various experiments a gaseous mixture will be found with which the blood is in equilibrium. For such determinations an aerotonometer is used. Because fish blood is somewhat difficult to handle and because of the small amounts that are usually obtained the aerotonometer has not been found practicable. Another method has been devised which is very similar in principle to that of the aerotonometer although absolute tensions can not be determined, but rather differences in tensions. However, the results are definite and strictly comparable.

A diagram of the apparatus used is shown in Fig. 7. An equilibration chamber *C*, opening at one end through a single way stopcock, 1, and on the other through a three-way stopcock, 2, was connected to a levelling bulb, *L*, by rubber pressure tubing. A vertical glass tube, *G*, was inserted in the rubber tube to make visible the column of mercury within. The equilibration chamber could be shifted so that the stopcock, 2, was down (position A) or so that it was up (position B). *T* represents the position of a test tube during the experiment and *B* a constant temperature water bath surrounding the test tube.

Gas from the swimbladder of the bass which was known to have a high oxygen tension was drawn into the equilibration chamber, *C*, through a hypodermic needle, by filling the entire apparatus with mercury previously and by lowering the levelling bulb, *L*, with the equilibration chamber in position *B* until the mercury column could be seen in the tube *G*.

Blood was drawn from the dorsal aorta of the bass under par-

affin oil, care taken that the blood did not at any time come in contact with the air. The blood was oxalated to prevent clotting. The Van Slyke method was used for the determination of the blood gases (Van Slyke and Stadie) (1921). Usually two

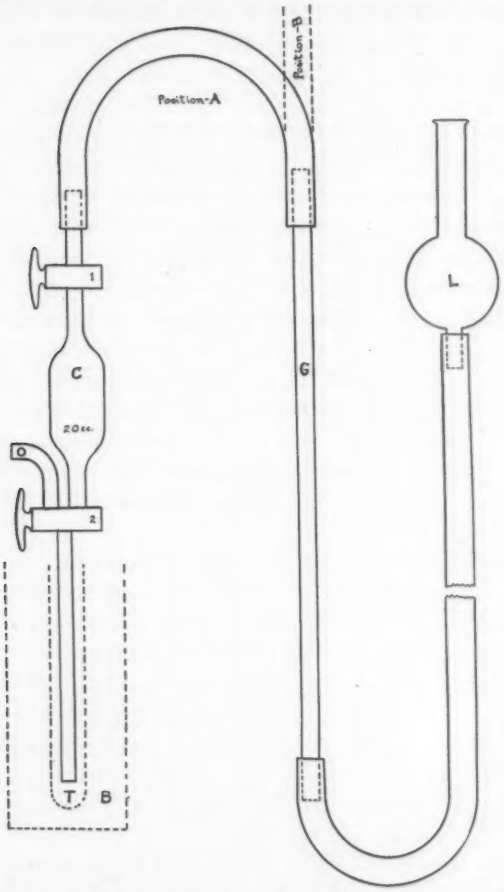


FIG. 7. Blood gas and swimbladder gas equilibration apparatus.

determinations were made to insure accuracy. Following these determinations, the test tube containing the blood was inserted in the position shown in Fig. 7, *T*, (position A). Oil was then drawn up into the tube, through O, to displace the air. The

stopcock *2* was then turned to connect the equilibration chamber with the test tube. The levelling bulb was then lowered and the blood in the test tube drawn up into the equilibration chamber *C*. The stopcocks were then closed and the chamber shaken for several minutes until the gases in the blood and the swimbladder gases came into equilibrium with each other. The blood was then allowed to run back into the test tube. Another series of Van Slyke determinations were made. Constant temperatures were maintained throughout the experiments. By this method differences of tensions could be determined. For if the analyses showed an increase or decrease in the determinations before and after equilibration of the blood with the swimbladder they would denote a greater or lesser tension of the gases. The results of the determinations are shown in Table VIII.

TABLE VIII.

RESULTS OF EQUILIBRATION OF FISHES' BLOOD WITH SWIMBLADDER GAS.

Air was previously removed from the swimbladder in order to stimulate secretion. The gas composition recorded represents the percentages at the time the blood was removed from the fishes.

No.	Gases in Swimbladder.		Normal Blood, Vol. per cent.		Blood after Equilibration, Vol. per cent.		Difference, Vol. per cent.	
	Per Cent. O_2.	Per Cent. CO_2.	O_2.	CO_2.	O_2.	CO_2.	O_2.	CO_2.
Bass								
1	46.0	5.54	3.08	20.9	5.74	20.9	+2.66	0
2	30.0	3.06	4.74	22.2	6.47	22.8	+1.73	+0.6
3	32.7	3.40	4.60	21.8	6.41	21.8	+1.81	0
4	41.5	4.30	3.84	22.0	6.16	22.4	+2.32	+0.4
Average	37.5	4.08	4.07	21.7	6.20	22.0	+2.13	+0.25
Carp								
1	4.92	3.00	4.75	8.85	3.07	8.6	−1.68	−0.25
2	7.40	3.32	4.40	12.4	4.82	12.4	+0.42	0
3	1.80	0.75	4.68	9.15	3.02	9.15	−1.66	0
4	5.35	5.80	2.84	10.2	3.05	10.8	+0.21	+0.6
5	6.10	3.87	4.62	11.1	4.88	11.1	+0.26	0
Average	5.11	3.35	4.26	10.34	3.77	10.41	−0.49	+0.07

It will be observed that in experiments with the bass a greater tension of oxygen existed in the swimbladder than in the blood, indicating that an active secretion of oxygen takes place.

In the carp, however, which has a duct from the swimbladder opening into the pharynx, no such tension difference could be detected. Physical diffusion probably accounts for the passage of gases to and from the swimbladder in this fish.

In the normal bass living at the surface, where little or no depth changes are taking place, the percentage of the gases in the swimbladder is probably controlled by simple diffusion. For when determinations were made on such bass the tension of the swimbladder gases and the blood appeared to be the same. It is only when changes such as pressure made a rapid increase of gases in the swimbladder necessary that secretion operated.

Discussion.

The results of this investigation show that in the swimbladder of fishes an active secretion of gases exists, especially of oxygen. There apparently is a regulatory mechanism by which fishes can adjust their specific gravity to that of the environment, thus enabling them to maintain themselves at any particular level with a minimum expenditure of energy. What is this mechanism? Gas secretion has been an extremely controversial subject, especially when reference is made to the lung of the higher vertebrates and the swimbladder has been considered by many to be the homologue of the lung.

It is quite apparent that the gases of the swimbladder are derived from the blood. Oxygen plays the more active rôle in this secretion and nitrogen, which is more inert, is more passive. This at first may seem strange, but if a gas is to be secreted from the blood or absorbed by the blood the advantages are obvious. Oxygen can be easily obtained from oxy-hemolobin and can be used up either by combination with reduced hemoglobin or by oxidation of some reducing substance.

In the mechanism by which the swimbladder derives its oxygen two provisions are necessary: first, the provision for bringing the quantity of oxygen which is required; second, the process by which the oxygen is transferred from the capillary blood vessels to the swimbladder.

The Swimbladder Gland.

If one examines the structure of the swimbladder of certain fishes, at one region or another he will see a glandular structure consisting of a thickened layer of epithelial cells. Below this a more or less conspicuous area, red in color, is visible. This "red body" and its contiguous epithelium is known as the swimbladder gland. To Johannes Müller (1842) may be ascribed the discovery of its true glandular nature. The mass of red blood vessels of the red body, which has for years attracted the attention of anatomists, was discovered by Redi (1684) and described by him. Since his time it has been known by the name of the *rete mirabile.*

Woodland (1911) made a very careful study of the structure of swimbladder glands of many species of fishes. He describes the gland as having two separate parts—the glandular epithelium or gas gland and the *rete mirabile.* The rete may or may not be contiguous with the epithelium. The glandular epithelium may be a single layer or many layered. Woodland found that the arterioles passing to the gland break up into capillaries which come into intimate contact with corresponding venous capillaries from the venules coming from it. There was a free anastomosing of the capillaries on the arterial side with those on the venous side.

The capillaries were regarded for a long time as passive endothelial filters with no muscle and therefore possessing no independent contractility. Krogh (1920) has, however, proved that the capillaries do possess the ability to contract. He has also shown that when many substances are introduced into the blood stream they have marked action on capillaries. Urethane produces extreme dilatation of the capillaries and stasis. Oxygen deficiency leads also to a marked capillary dilatation. Krogh has adduced evidence, from an admirable series of experiments, that the capillaries are normally kept tonically contracted by a substance present in the blood stream. He believes that the pituitary hormone is the substance that maintains the capillary tone, and is present normally in concentration of about one part in a hundred million or less. Krogh also maintains that oxygen,

carbon dioxide and probably all crystalloids pass through the capillary walls by purely physical diffusion.

It hardly seems necessary to go further into a discussion of the many experiments that have been performed in this field. It is apparent that when certain metabolic products are thrown into the blood stream because of a heightened metabolism of a gland an increased vascular dilatation ensues, producing a heightened flow of blood to that gland. What application can be made of this process in explaining the mechanism of gas transference in the swimbladder of fishes? Before attempting to answer that question it will perhaps be fitting to mention another means by which the gland is able to regulate the supply of oxygen in proportion to the needs.

A study was made of the structure of the swimbladder gland of the yellow perch by the writer. It is located on the ventral side of the swimbladder wall near the anterior end. The rete mirabile is seen as a group of small fan-shaped tufts formed by an artery and a vein. A thickened glandular mass of epithelium surrounds the "red body." Micro-photographs (Plate I) also show the mass of capillaries and their terminations in the epithelial cells of the gland.

The blood supply is arterial, coming from either the aorta or the coeliac axis, and in some fishes different portions receive blood from both sources. In the walls of the bladder the arteries break up into the networks of the rete mirabile. From the rete the blood passes to the body veins—postcardinal, hepatic, or vertebral. It seems reasonable to conclude that this remarkable distribution of the blood vessels furnishes an adequate means of supplying the swimbladder with blood for the purpose of secretion.

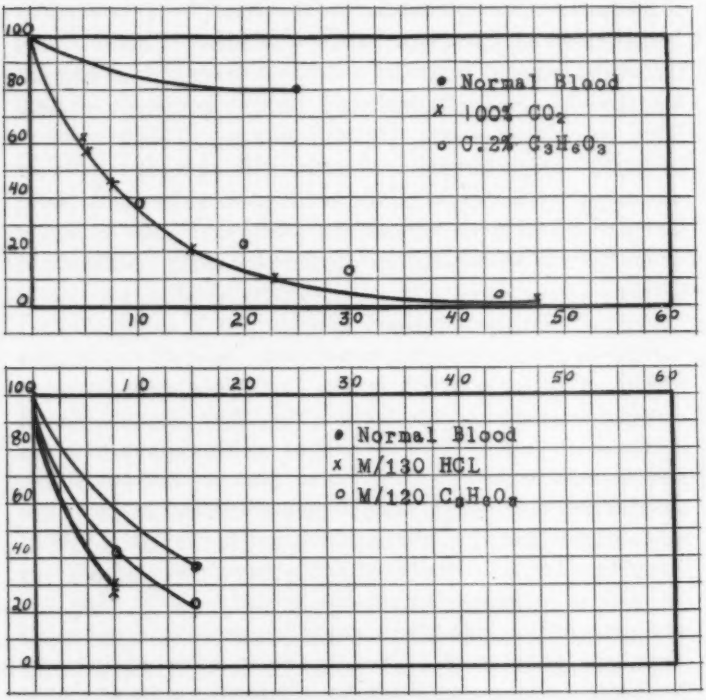
The second provision—the process by which the oxygen is transferred from the capillary blood vessels to the swimbladder is a more strictly physiological problem. It is evident that the oxygen is first given to the cells of the rete mirabile and then passes out the glandular cells into the swimbladder cavity. This transference of oxygen from the red cell involves a chemical breakdown of the oxy-hemoglobin in the first place, and, following this liberation of oxygen, a diffusion of gas occurs between

the blood and the cells. In such a physiological system it is necessary that a definite amount of blood should pass through an organ; it must give a definite oxygen tension to the organ and in so doing becomes reduced. The amount of that reduction varies with the need of any particular organ. The question arises—is there evidence that each or any organ of the body is so far master of its own metabolism that it can force the vascular system to give it the oxygen which it requires. Gaskell (1880) stated this question years ago. Evidence has ever been increasing since his time. Gaskell and other more recent workers have shown that certain metabolic products, especially acids, including carbon dioxide and lactic acid, have the power of distending blood vessels. Dale and his colleagues (1911) have also shown that beta- iminazolylethylamine, a body so closely bound up with the physiology of protoplasm that it is liberated by the splitting off of carbon dioxide from histidine, produces a powerful dilatation of the blood vessels.

Barcroft (1914) and other investigators have shown that the affinity of hemoglobin for oxygen is very sensitive to small changes in acidity or alkalinity. The effect of an increased acidity is to lessen the concentration of the oxygen which is held in solution by the hemoglobin. Barcroft has also shown that the influence of acids depends upon the change in the hydrogen ion concentration which they cause in the blood. Bohr (1896) found experimentally that when the oxygen tension is low, an increase in the carbon dioxide tension tends to dissociate oxy-hemoglobin. Since these conditions prevail in the capillaries, the presence of carbon dioxide in increased amounts facilitates the liberation of oxygen. Barcroft maintains that it is the "reaction" of the blood that has the effect of varying the dissociation of oxygen from oxy-hemoglobin. The more acid the blood contains, or the more acid the "reaction" due to the increased amounts of lactic acid or an increased tension of carbon dioxide, the more readily does the oxy-hemoglobin undergo dissociation.

Two experiments described by Barcroft show that the addition of acids greatly accelerates the reduction of blood and that the concentration of the acids necessary to produce approximately

equal effects are those in which they produce equal increments in the hydrogen ion concentration. In Fig. 8 it will be seen that the time taken for mammalian blood containing 0.2 per cent. lactic acid to be reduced by nitrogen, and for blood without lactic acid to be reduced by 100 per cent. carbon dioxide, are indistinguishable. But blood containing either of these solutes requires



FIGS. 8 AND 9. Ratio of reduction of blood with a uniform stream of oxygen free gas. Percentage saturation is shown vertically. Time in minutes is shown horizontally. Fig. 8 shows a comparison with normal blood, with blood containing lactic acid, and with blood reduced by carbonic acid instead of nitrogen. Fig. 9 shows the approximate equality of *M*/130 HCl and *M*/120 lactic acid in their effects. (From Barcroft, 1914.)

about one tenth the time necessary for the same reduction without acids. The blood is reduced from 100 per cent. saturation to 80 per cent. saturation, in about two minutes with the acids present, and about twenty-two minutes with the acids absent.

In Fig. 9 it will be seen that normal blood is reduced (the bubbling was more rapid and the temperature higher) from 100 per cent. to 35 per cent in fifteen minutes, while when it contained $M/130$ HCL and $M/120$ lactic acid the same reduction was effected in about six and one half and seven and one half minutes respectively. Thus it becomes evident that the "reaction," namely the hydrogen ion concentration of the blood effects the reduction of the blood; the amount of dissociation of the oxygen from the oxyhemoglobin is in proportion to the increase in the hydrogen ion concentration.

How may these facts be correlated with the conditions found in the swimbladder of fishes? The abundant supply of blood furnished to the swimbladder by means of the rete mirabile has already been described. The question arises—is the gland which includes the rete mirabile able to force the vascular system to give it the oxygen which it may require in order to maintain a pressure in the swimbladder equal to that at the depth to which a fish is subjected? Does it perform this function by secreting substances causing a dilatation of the blood vessels of the rete mirabile, thus producing an increased flow of blood to the gland, or do these substances accelerate the reduction of the oxy-hemoglobin, or is it a combination of both these processes?

An attempt was made to determine the hydrogen ion concentration changes that might take place in the swimbladder. In making the tests fish were quickly removed from the aquaria and the spinal cord severed just posterior to the brain. The body cavity was opened, the swim bladder gland raised by forceps and cut free from the body, as little time elapsing as possible during the operation. Care was taken not to get any of the body secretions or fluids on the gland. The gland was then placed in a small vial containing about five cubic centimeters of distilled water, which was free from carbon dioxide, which had been previously weighed. The vial was again weighed and the weight of the gland computed. After standing for a definite length of time (see Tables IX. and X.) colorimetric determination of the hydrogen ion concentration was made. The vial was only slightly agitated during the period of standing, in order to keep the gland intact. Three cubic centimeters were pipetted off for

the determinations. By this method the changes brought about in the hydrogen ion concentration by substances present in the distilled water were mainly dialyzed from the gland. It is only those substances which do dialyze from the gland that are of concern in this problem. In the first series of experiments the yellow perch was used and a definite amount of gas was removed from the swimbladder (five cubic centimeters) by methods already described in the gas withdrawal experiments, page 83. Care was taken not to injure the fishes more than was absolutely necessary. A very small needle was used and the puncture was made at the opposite end of the swimbladder from the gland.

TABLE IX.

DETERMINATION OF THE pH OF THE SWIMBLADDER GLAND OF NORMAL PERCH.

The gland was allowed to dialyze in 5 c.c. of distilled water free from carbon dioxide, and 3 c.c. was pipetted for the colorimetric determination.

No.	Weight of Gland.	Time of Dialysis.	pH.
1.....	0.215 gms.	30 min.	7.1
2.....	0.242 "	40 "	6.9
3.....	0.156 "	30 "	7.1
4.....	0.201 "	30 "	7.1
5.....	0.253 "	30 "	7.0
6.....	0.194 "	30 "	7.1
Averages.....	0.210 gms.	32 min.	7.05

TABLE X.

DETERMINATIONS OF THE pH OF THE SWIMBLADDER GLAND OF PERCH FOLLOWING THE WITHDRAWAL OF 5 C.C. OF THE GAS FROM THE SWIMBLADDER.

A similar amount of the carbon dioxide free water was used as in the determinations with normal fishes. The "duration of stimulus" of the decreased internal pressure indicates the time between the withdrawal of the gas and the removal of the gland from the fishes.

No.	Duration of Stimulus.	Weight of Gland.	Time of Dialysis.	pH.
1.....	45 min.	9.287 gms.	30 min.	6.4
2.....	2 hrs.	0.285 "	40 "	6.4
3.....	2 "	0.178 "	40 "	6.3
4.....	2 "	0.242 "	30 "	6.4
5.....	2 "	0.257 "	30 "	6.3
6.....	2 "	0.251 "	30 "	6.4
Averages.....		0.250 gms.	33 min.	6.38

Autopsies were made to ascertain the extent of the injury due to the puncturing and in no case was there any apparent evidence of injury to the gland.

As a control in some cases the swimbladders of normal fish were punctured with a sewing needle approximately the size of the hypodermic needle. No gas was removed. The results are shown in Tables IX. and X.

In the second series of experiments fishes were subjected to high pressures for several hours. The apparatus as described previously for pressure experiments, page 91, was used. The pressure in the apparatus registered from thirty-eight to forty-five pounds per square inch. The pressure was applied gradually and removed gradually. The results of these experiments are shown in Tables XI and XII.

TABLE XI.

DETERMINATIONS OF THE pH OF THE SWIMBLADDER GLAND OF NORMAL PERCH.

The gland was allowed to dialyze as in the preceding experiments; the same amounts of the carbon dioxide free water were used.

No.	Weight of Gland.	Time of Dialysis.	pH.
1	0.160 gms.	30 min.	6.9
2	0.535 "	30 "	6.8
3	0.310 "	30 "	7.0
4	0.316 "	20 "	7.0
5	0.305 "	10 "	7.0
6	0.308 "	30 "	7.0
7	0.333 "	30 "	6.9
8	0.314 "	30 "	7.0
Average	0.324 gms.	26 min.	6.96

These determinations show that the hydrogen ion concentration of the swimbladder gland increases when the fish are placed under pressure or when the pressure within the swimbladder is diminished. When five cubic centimeters of the gas was removed from the swimbladder the hydrogen ion concentration of the glands averaged 6.38 as compared with 7.05 of the controls. When fish were subjected to an increased pressure the hydrogen ion concentration of the glands averaged pH 6.64 as compared with pH 6.96 of the controls.

The swimbladder gland consists of connective tissue, a bed of capillaries, and a thick layer of epithelium (Plate I). Increase

TABLE XII.

DETERMINATIONS OF THE pH OF THE SWIMBLADDER GLAND OF PERCH SUBJECTED TO AN INCREASED PRESSURE.

These were made similar to those described in Table XI. The "duration of stimulus" indicates the time to which fishes were subjected to external pressure.

No.	Duration of Stimulus.	Pressure lbs./ sq. in.	Weight of Gland.	Time of Dialysis.	pH.
1.....	40 min.	38	0.122 gms.	30 min.	6.8
2.....	40 "	38	0.205 "	30 "	6.8
3.....	140 "	45	0.194 "	30 "	6.4
4.....	140 "	45	0.210 "	30 "	6.9
5.....	6 hrs.	45	0.392 "	30 "	6.3
6.....	6 "	45	0.405 "	30 "	6.3
7.....	6 "	45	0.745 "	30 "	7.0
8.....	6 "	45	0.330 "	30 "	6.6
9.....	6 "	45	0.365 "	30 "	6.7
Average ..	4 hrs.	43	0.331 gms.	30 min.	6.64

in the acidity is evidently produced in the epithelial cells and acid dialyzes into the capillaries. Two things may be produced in the capillaries: (1) an increase in the calibre of the capillaries; (2) a more complete dissociation of oxygen from oxy-hemoglobin. In either case more oxygen would be furnished to the gland. By increasing the calibre of the blood vessels more blood is brought to the gland and by a more complete oxygen dissociation a greater oxygen tension results. It is not possible to account for the greater oxygen tension in the swimbladder by the first means; by the second, however, where the local tension of oxygen might be so high, it is possible to see how the oxygen tension in the swimbladder might be considerably higher than in the general arterial system. If this hypothesis be correct it is easy to undersrand the purpose of the great mass of capillaries. The function on the thick layer of epithelium is also explained. Just what the nature of the effective acid is can not be stated at present. Perhaps further investigation will reveal its character.

Another observation which seems to support the view just proposed relates to the change in size which the gland undergoes. It is noticeable that there is an increase in the size of the swimbladder glands that have been stimulated. They also appear redder in color. The increase in size is evidenced also by the increase in weight shown in Tables IX.–XII. The average

weight for the normal glands of perch was 0.267 grams as compared with 0.290 grams for glands of perch subjected to pressure stimuli. A considerable variation occurs in the cutting of the glands free from the swimbladder, and these figures are not absolute quantitative comparisons; nevertheless they may be taken as indicative.

It is probable that afferent nerves perhaps located in the walls of the swimbladder that are sensitive to changes in pressure aid in the regulation of the specific gravity of the fish. Bohr (1893) showed that the vagus exerts a control over the gas secretion in the swimbladder. Its control is very likely a matter of stimulating the swimbladder gland to secrete. For example when a perch sinks into deeper water the pressure outside increases. In order to maintain its equilibrium, the perch must increase the amount of gas in the swimbladder. It does this by taking oxygen from the blood. When the fish rises in the water oxygen must be re-absorbed by the blood. When the fish is not changing its depth appreciably the gas content of the swimbladder is probably maintained by simple physical diffusion of the gases from the blood, but when a greater difference of pressure is met with the secretory process comes into play.

The mechanism by which those fishes which have a duct leading from the swimbladder maintain their equilibrium in the water has not been studied in detail. Whether the process of gas diffusion is fundamentally different in such fishes can not be stated. Further investigation will be made using such fish as carp, dogfish, and trout. In all events some modifications may be expected for no such highly specialized organ as the rete mirabile exists in these forms.

The writer's hypothesis of the mechanism by which the swimbladder is able to regulate the specific gravity of the fish, is not intended as a complete explanation of all the processes involved in gas secretion. It does however offer a chemical and physical explanation of a very controversial matter. The swimbladder of fishes offers unusual opportunities for the study of physiological processes accompanying gas secretion and no doubt new facts may be learned from them of fundamental biological importance.

Summary.

1. The normal gaseous content of the swimbladder of fresh-water fishes near the surface is approximately that of the atmosphere. The composition varies with the species, pressure, temperature, amounts and kinds of dissolved gases, and with the seasons of the year.

2. When fishes are placed in water containing little or no oxygen the oxygen in the swimbladder diminishes; indicating that the swimbladder may act as a reservoir on which the blood may draw for oxygen in times of need. A perch is enabled to go into water of low oxygen content without asphixiation.

3. The effect of increased pressure in the surrounding water is to increase both the percentage of oxygen and carbon dioxide in the swimbladder.

4. If carbon dioxide is increased in the medium in which perch are living, the volume of the fishes is changed and the fishes automatically rise in the water. This response would be of adaptive value, causing the fish to move out of deeper water containing larger amounts of carbon dioxide into the safer zones above.

5. The primary function of the swimbladder of most of the fresh-water fishes is hydrostatic.

6. Perch apparently possess no voluntary muscular control over the size of the swimbladder.

7. Under conditions where high oxygen percentages were found in the swimbladder, a higher tension of the gases existed than in the blood. This indicates an active secretion.

8. Under conditions where fishes are not changing their depth rapidly, the gases in the swimbladder are probably kept constant by simple diffusion of gases from the blood.

9. A "rete mirabile" partially surrounds the walls of the swimbladder and furnishes a rich supply of blood. It is apparently the means by which the gases are transferred from the blood to the swimbladder.

10. The mechanism by which gas is secreted into the swimbladder can apparently be explained on a chemical and physical basis. The writer's experiments show that the hydrogen ion concentration of the swimbladder gland is increased by external stimulation. This indicates the secretion of a substance by

the gland which may aid in the secretion of gases into the swimbladder. The apparent secretion of oxygen is believed to be brought about by (*a*) an increased flow of blood because of the dilatation of the capillaries and (*b*) an increased tension of the oxygen due to the local dissociation of oxygen from oxy-hemoglobin.

11. The swimbladder is a mechanism which enables the fish to actively maintain its stability in the midst of changing external conditions.

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PLATE I.

Photo-micrographs of the swimbladder gland showing the glandular epithelium, *g.e.*; and the rete mirabile, *r.m.* From perch.

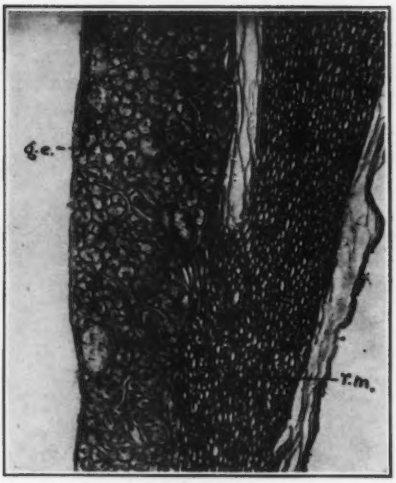

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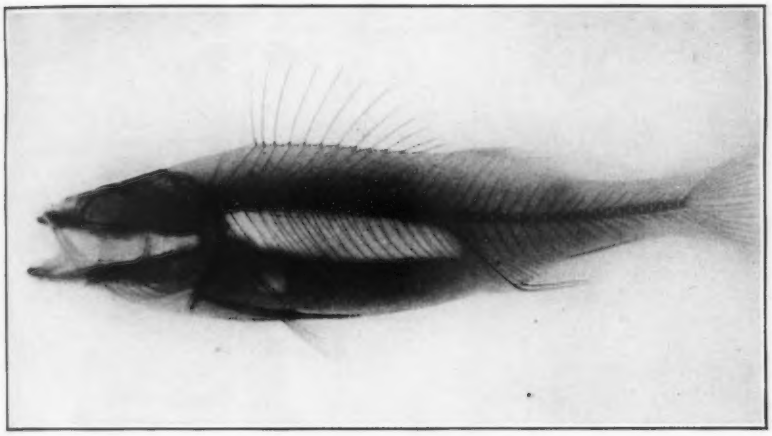
PLATE II.

X-Ray of Yellow Perch.
X-Ray of Largemouth Black Bass.



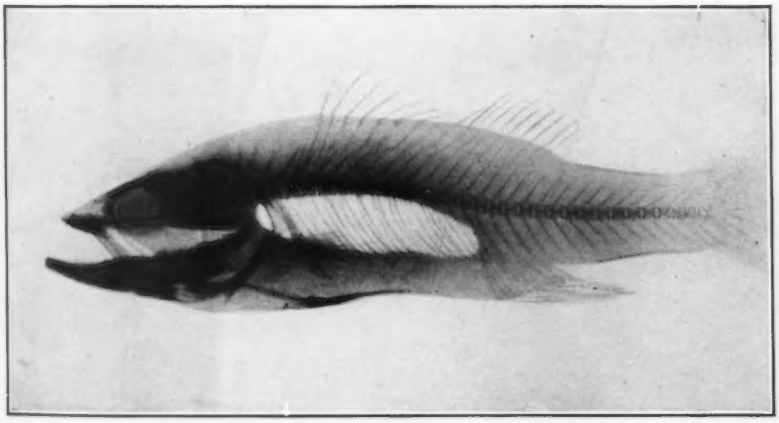


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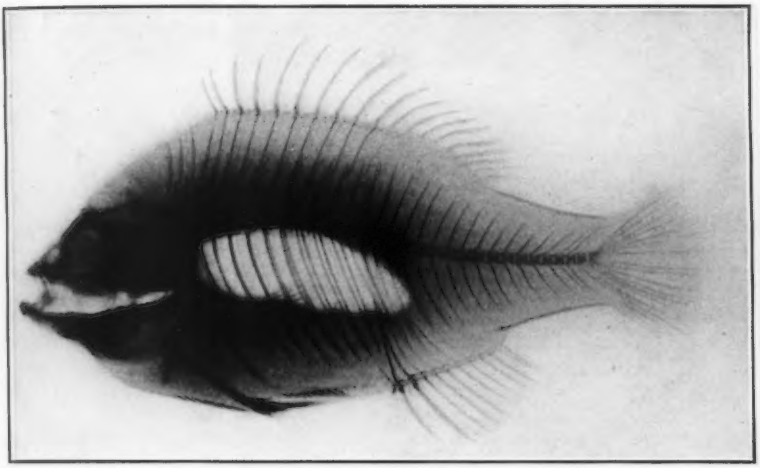
PLATE III.

X-Ray of Sunfish.
X-Ray of Blue-gill.



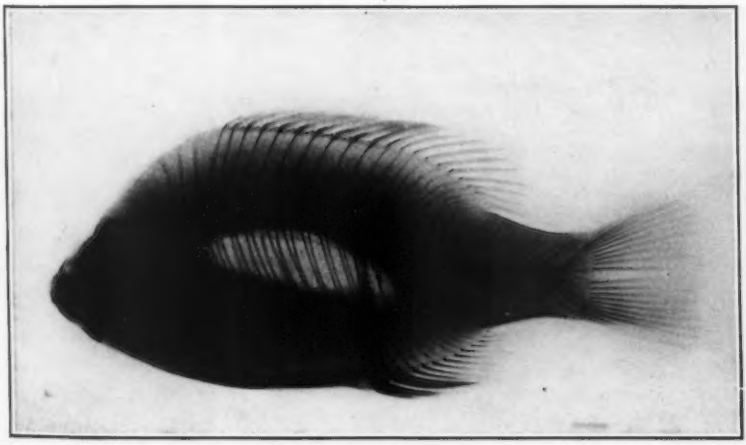


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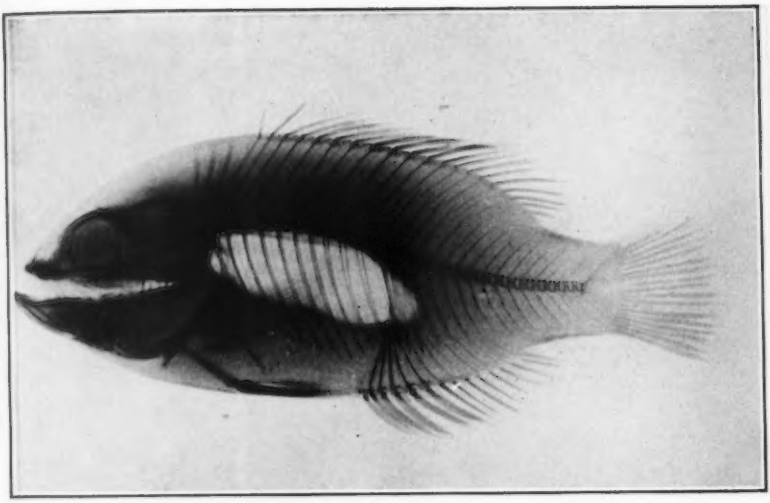
PLATE IV.

X-Ray of Rock-Bass.
X-Ray of Bull-head.



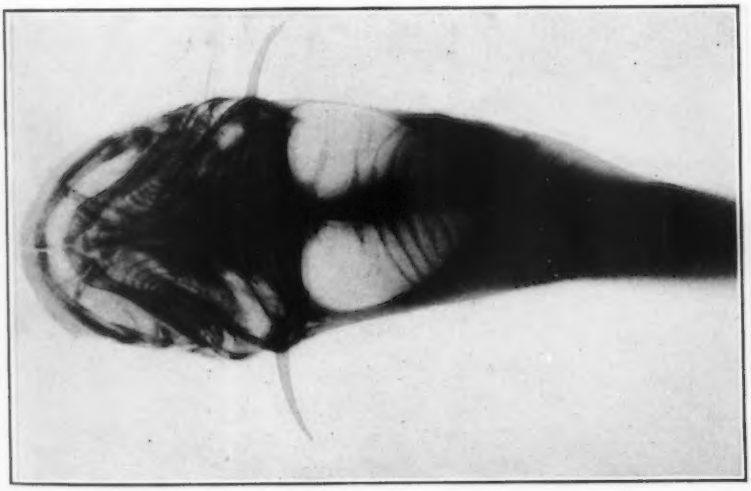


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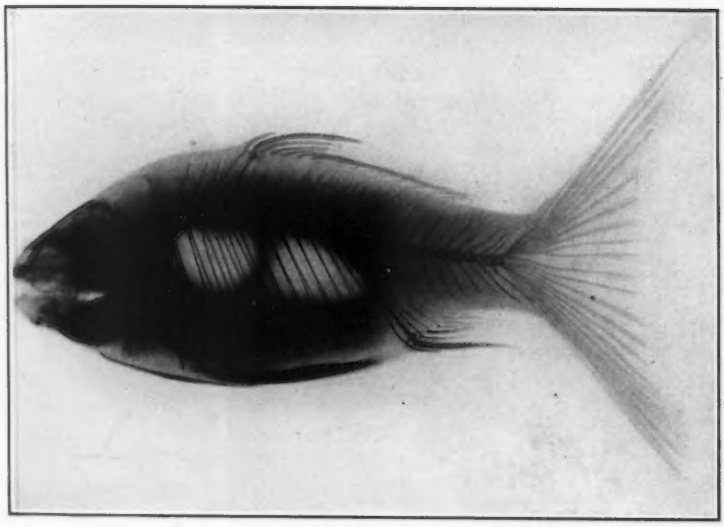
PLATE V.

X-Ray of Gold-fish.



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